strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C, and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product.

D1 Cont

9. (Twice Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

D2

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase has proofreading 3'-5' exonuclease activity during DNA primer extension over a template, such that the DNA polymerase functions to excise mismatched nucleotides from the 3' terminus of the DNA strand at a faster rate than the rate at which the DNA polymerase functions to remove nucleotides matched correctly with nucleotides of the template, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(ii) effecting cycle primer extension reaction(s) at a temperature below about 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their

D2

corresponding analogs and thereby produce a sequence-specific amplification product.

22. (Amended) A method for extending an oligonucleotide primer or a pair of oligonucleotide primers using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature of about 70°C, comprising the step of mixing a template DNA with a primer or a pair of primers and a natural or a modified form of a moderately thermostable DNA polymerase from an organism selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, in a solution containing between about 10% and about 20% (v/v) glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, under conditions that the cycle reaction temperature fluctuates between a melting temperature of about 70°C and an annealing temperature of about 37°C, so that the DNA polymerase repeatedly extends the primer or pair of primers to produce a sequence-specific amplification product.

D3

30. (Amended) A method for extending an oligonucleotide primer annealed to a DNA template for direct cycle sequencing of in vitro amplified double-stranded DNA products without prior isolation or purification, using an enzymatic cycle primer extension reaction at temperatures between about 45°C and about 65°C, and a melting temperature below about 80°C, comprising the steps of:

D4

(i) mixing diluted crude amplified reaction product with an excess amount of a sequencing primer, the four standard ddNTP terminators or their corresponding analogs, a native or modified form of a moderately thermostable DNA polymerase selected from the group consisting of *Bacillus stearothermophilus*, *Bacillus caldotenax* and *Bacillus caldolyticus*, wherein the DNA polymerase

during dye-labeled terminator automated DNA cycle sequencing reduces the innate selective discrimination against the incorporation of fluorescent dye-labeled ddCTP and fluorescent dye-labeled ddATP, a suitable concentration of dNTPs, and a composition comprising a buffer in a solution containing about 10% to about 20% of glycerol, ethylene glycol, or a mixture of glycerol and ethylene glycol, and

(ii) affecting cycle primer extension reaction(s) at a temperature below 80°C for a sufficient number of times to extend the sequencing primer molecules to desired lengths terminated specifically by ddNTPs or their corresponding analogs and thereby produce a sequence-specific amplification product.

REMARKS

Reconsideration and allowance of the subject application are respectfully requested.

Claims 1 and 9 are amended to address the Examiner's objections under 35 U.S.C. §102(b) regarding the Fuller and Ruano references. Claims 22 and 30 are amended to address the Examiner's objections under 35 U.S.C. §102(b) regarding Hong et al. (U.S. Patent 6,165,765) and Hong et al., (6,485,909). Support for these amendments can be found throughout the specification, for instance, at page 4, line 6 – page 7, line 8; page 9, line 9 – page 12, line 23. No new matter is introduced, and entry and full consideration is requested. Upon entry of this amendment, claims 1-11 and 18-35 will remain pending.

In the Office Action dated February 5, 2003, claims 1-8 were rejected under 35 U.S.C. §102(b) as anticipated by Fuller (U.S. Patent 5,432,065). Claims 9-11 were rejected under 35 U.S.C. §103(a) as obvious over Fuller in view of Ruano (U.S. Patent 5,427,911). We traverse these rejections for the following reasons.

In our last Amendment mailed October 24, 2002, we noted that, while Fuller mentions a glycerol level of 10 – 50% v/v and an ethylene glycol level of 20% v/v, there is no data whatsoever to support this, and, in fact, there is evidence in the prior art that applying the teachings of Fuller does not generate amplification products that are